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REVIEW ARTICLE

End-points for drug treatment in NASH

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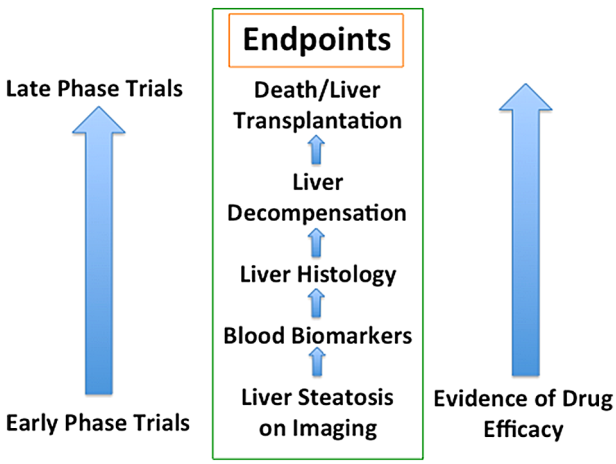
Abstract

Non-alcoholic steatohepatitis (NASH) is an increasingly common cause of cirrhosis, hepatocellular carcinoma, and liver-related death (LRD). Consequently, there is a critical need for effective drug therapy that improves clinically relevant end-points. Hepatic steatosis assessed by magnetic resonance imaging-proton density fat fraction is increasingly used in the early phase trials examining drugs with anti-steatotic effects. However, the prognostic significance of a reduction in steatosis is unknown, and thus, phase 3 trials require a histological end-point of NASH resolution without fibrosis progression. Nonetheless, it is not clear whether this end-point which requires a liver biopsy reflects a better prognosis. Thus, conditional drug approval currently requires long-term follow-up to demonstrate reduction in 'harder' end-points of cirrhosis, liver transplantation, and LRD. Currently, there is an essential need to develop accurate non-invasive markers that are dynamic to drug-induced changes in underlying disease severity and prognosis to utilize as surrogate end-points for clinical trials in NASH.

Graphical abstract



Keywords Non-alcoholic fatty liver disease · Clinical trials · Cirrhosis · Histology · Non-invasive fibrosis models · MRI

Introduction

Liver-related death (LRD) due to complications of cirrhosis or hepatocellular carcinoma (HCC) is uncommon in patients with non-alcoholic fatty liver disease (NAFLD) within the general population [1]. However, due to the high prevalence of NAFLD, the associated health burden is significant and increasing [2], and effective treatments are required. Lifestyle intervention is the first-line treatment for NAFLD;

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however, it has limited long-term success, and thus, effective drug treatments are required. Currently, there is an intense activity in the development and testing of new agents for NAFLD; however, the challenge remains of who is most appropriate to receive expensive and potentially harmful drug treatment and how to assess treatment efficacy. To this end, the determination of appropriate treatment end-points for clinical trials remains a key question in the search for effective therapies for this common disease.

Patient inclusion criteria for drug treatment

Before defining a therapeutic end-point, it is important to determine what factors determine need for drug treatment, and thus characterize the baseline status from which efficacy will be assessed. To this end, liver histology is currently the strongest predictor of overall and liver-related morbidity and thus eligibility for drug treatment. NAFLD is a histologically heterogenous condition ranging from simple steatosis to non-alcoholic steatohepatitis (NASH) to cirrhosis. Those with simple steatosis are at low risk (0–2%) of developing cirrhosis over 1–2 decades [3, 4]. Subjects with NASH have a higher rate of fibrosis progression, with a meta-analysis of paired liver biopsies, demonstrating that the average time to progress one fibrosis stage was 7 years in patients with NASH versus 14 years in those without. Correspondingly, patients with NASH are more likely to develop end-stage liver disease than non-NASH patients with one cohort study demonstrating 10% versus 0% over an average of 13 years [5]. Among patients with or without NASH, however, fibrosis stage is the dominant predictor of outcomes, with risk of liver-related events increasing progressively once moderate (stage 2) fibrosis is reached [6]. The risk of a liver-related decompensation or HCC reaches 4%, 7–15%, and 30–35% in patients with moderate (F2), advanced fibrosis (F3), or cirrhosis (F4) respectively, after 10 years [6, 7]. Therefore, patients with NASH and moderate to advanced fibrosis or cirrhosis are at most risk and are being targeted for clinical trials.

Treatment goals and hard end-points

The treatment goal for physicians managing patients with NAFLD is to prevent death, reduce disease-related morbidity, and improve quality of life. Therefore, the evaluation of efficacy for new drug treatments should reflect these end-points. The major cause of death in patients with NAFLD is cardiovascular disease (CVD), and so, ideally, an effective treatment will ameliorate CVD risk factors. Nevertheless, the current drug targets are primarily directed at reducing liver steatosis, injury, and fibrosis, and thus, a reduction in liver-related mortality is a more rational therapeutic end-point. However, the liver-related death rate is low, being 1.7% over 7 years among community-based NAFLD patients, and 1.7% per annum among compensated NASH cirrhosis patients [7, 8]. Demonstrating efficacy for the end-point of liver-related mortality is, therefore, prohibitively expensive and time-consuming, requiring 1000s of patients to be followed over many years.

Liver decompensation is associated with symptoms, reduced quality of life, hospitalization, and cost. Liver decompensation also heralds a significant increase in mortality risk in NAFLD patients, being approximately 50% at 2 years [9]. Thus, the development of liver decompensation is a clinically relevant end-point; however, it also remains logistically challenging due to its low rate of development.

Surrogate drug treatment end-points: histology

Given the logistical difficulties of demonstrating drug efficacy on hard clinical end-points such as hepatic decompensation or liver-related death, surrogate markers of these end-points are also accepted by regulatory bodies including the FDA and EMA. A surrogate is a marker that predicts clinical benefit on irreversible morbidity and mortality. Surrogate end-points currently used in clinical trials for NASH are outlined in Table 1 and Fig. 1. Importantly, surrogate measures may be misleading if off-target drug effects lead to adverse patient outcomes despite an improvement in the surrogate outcome [10].

The development of liver decompensation in NASH requires the presence of cirrhosis, and thus, the development of cirrhosis is a reasonable surrogate outcome. Notably, this may not be a reliable marker for the development of HCC which may occur in up to one-third of NAFLD patients in the absence of cirrhosis [11]. Current phase 3 trials including obeticholic acid and elafibranor involving 1000s of patients followed for up to 4 years, have a co-primary composite end-point of all-cause mortality, liver-related clinical outcomes, and the histological development of cirrhosis. Among patients with cirrhosis, the MELD score, CTP score, and hepatic-venous pressure gradient (HPVG) are prognostic of clinical decompensation, and have potential as candidate surrogate markers. However, they await validation as dynamic end-points that reflect changing prognosis in response to treatment among patients with NASH cirrhosis.

Fibrosis progression in NAFLD is characteristically slow, and so, the time taken to develop the surrogate end-point of cirrhosis will take numerous years. Due to the unmet need for effective pharmacotherapy for NASH, regulatory authorities have allowed surrogate histological end-points that change over a shorter time period,

Table 1 End-points for drug trials in NASH and comparison of methods of end-point evaluation. The majority of surrogate end-points have not been demonstrated to predict liver-related morbidity or mortality but may be useful in the early phase mechanistic studies

End-point	Validated for predicting liver morbidity or mortality	Method of evaluation	Gold standard of evaluation	Benefits of assessment method	Limitations of assessment method
Surrogate end-points					
Liver enzymes	No	Blood test	Gold standard	Simple, inexpensive	Not reflective of changes in fibrosis, very limited prognostic accuracy
Hepatic steatosis	No	MRI/MRS	Gold standard	Accurate, quantifies anti-steatotic effects in early phase mechanistic trials	Expensive, limited accessibility
		Ultrasound	Alternative	Accessible, inexpensive	Modest accuracy, operator variability
NASH	No	Liver biopsy	Gold standard	Direct measure of liver injury	Sample error, pathologist variability, invasive
		Blood biomarker (e.g., CK-18, Mac-2bp)	Alternative	Non-invasive, rapid	Modest accuracy, not validated to be dynamic to changes in NASH over time
Liver fibrosis and cirrhosis	Yes	Liver biopsy	Gold standard	Direct measure of liver fibrosis, Predictive of liver morbidity and mortality	Sample error, pathologist variability, invasive
		Elastography (e.g., fibroscan, MRE)	Alternative	Non-invasive, reproducible	Modest accuracy in determining changes in fibrosis over time
		Blood biomarker (e.g., NAFLD fibrosis score, FIB-4, ELF, Hepascore	Alternative	Non-invasive, reproducible, accessible	Modest accuracy, not validated to be dynamic to changes in fibrosis over time
Hard end-points					
Liver decompensation and liver-related death	Yes	Clinical	NA	Directly reflects goal of treatment	May not reflect off-target drug effect (e.g., cardiovascular mortality). Requires large study population with long duration of follow-up
All-cause death	Yes	Clinical	NA	Directly reflects goal of treatment	Requires large study population with long duration of follow-up

MRI/MRS magnetic resonance imaging/spectroscopy, *CK-18* cytokeratin-18, *Mac-2bp* Mac-2-binding protein, *MRE* magnetic resonance elastography, *ELF* enhanced liver fibrosis score

in order to facilitate accelerated or conditional approval for marketing. These surrogate end-points are: (1) resolution of NASH without worsening of fibrosis, and (2) one or more stage reduction in fibrosis without worsening of NASH [12]. Notably, these end-points have not been validated as predicting a clinical benefit (i.e., reduction in liver-related morbidity or mortality); however, they are considered to be 'reasonably likely' to predict clinical benefit based on epidemiologic, therapeutic, and pathophysiologic evidence [10]. Following conditional approval, the current FDA regulatory processes subsequently require the demonstration of benefit on a 'hard' clinical end-point (i.e., progression to cirrhosis, liver decompensation, overall death, and liver transplantation) for the final approval [13].

The histological resolution of NASH requires the disappearance of ballooning and absence or minimal lobular inflammation [12]. Notably, this is a departure from the previous histological end-points utilized in clinical trials which utilized improvement in the NAFLD Activity Score (NAS). The NAS is a composite score of steatosis (0–3), ballooning (0–2), and lobular inflammation (0–3), and was developed by the NASH Clinical Research Network for monitoring of NASH activity in clinical trials [14]. A more recent scoring

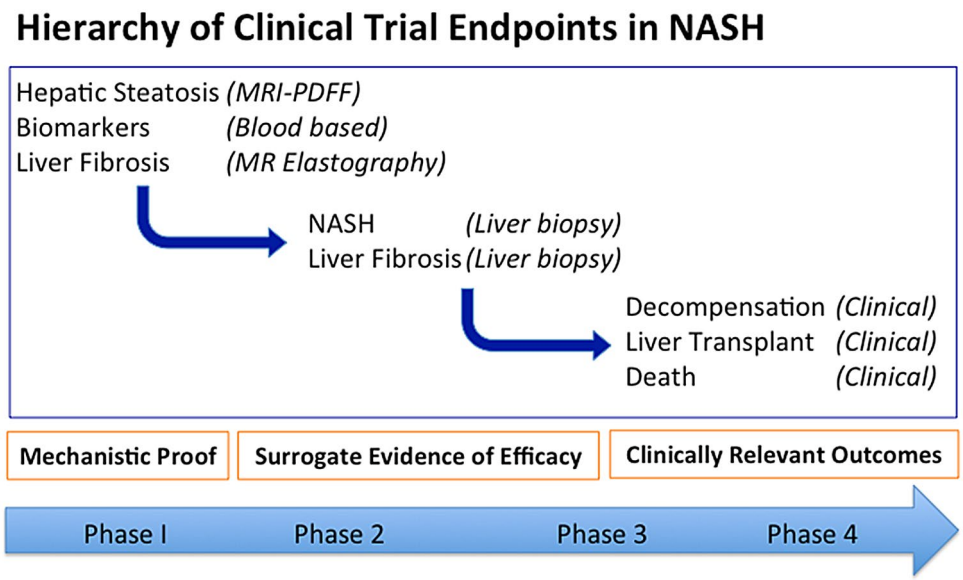

Fig. 1 Hierarchy of clinical trial end-points in NASH. Early phase I trials use end-points that reflect the mechanism of action such as MRI quantification of hepatic steatosis (for anti-steatotic drugs), blood biomarkers (for anti-inflammatory or anti-fibrotic drugs), or liver elasticity (for anti-fibrotic drugs). Phase 2 and 3 trials require surrogate end-points of efficacy such as histological improvements in NASH or fibrosis. Late phase trials require the evidence of clinically relevant hard end-points such as liver decompensation, liver transplantation, and liver or overall death. *MRI-PDFF* magnetic resonance imaging-proton density fat fraction, *MR* magnetic resonance

system (SAF score) combines steatosis, activity (consisting of ballooning and inflammation), and fibrosis, and defines NASH by the presence of lobular inflammation and ballooning. Neither the NAS nor SAF score has been demonstrated to be predictive of mortality or liver decompensation following adjustment for fibrosis stage. Nevertheless, the close pathophysiological link between NASH and fibrosis has lead its approval as a histological surrogate end-point.

Liver fibrosis is the dominant histological factor that is predictive of hard outcomes.

Thus, improvement in fibrosis would appear to be a reasonable surrogate histological end-point; however, its potential limitations have recently been raised [12]. For example, a drug which leads to a greater proportion of patients with one-stage fibrosis improvement but the same or greater proportion with progressive fibrosis when compared to a second drug, may be judged as being superior.

Surrogate drug treatment end-points: imaging

Hepatic steatosis

Hepatic steatosis is the defining histological feature of NAFLD. Phase I and IIa trials commonly use hepatic steatosis determined by highly accurate magnetic resonance techniques as a primary efficacy end-point, particularly when examining agents whose mechanism is expected to be anti-steatotic. For example, GS-0976, an inhibitor of the rate-limiting enzyme involved in de novo lipogenesis (acetyl-coenzyme A carboxylase), was predicted to have an anti-steatotic effect [15]. This efficacy end-point, however, may be less relevant for therapeutic agents which target hepatic fibrosis or inflammation. For example, selonsertib is an inhibitor of apoptosis signal-regulating kinase-1, and was predicted to reduce liver injury and fibrosis rather than steatosis. Consequently, a phase II trial demonstrated improvement in hepatic fibrosis but not steatosis which lead to the commencement of an ongoing phase III trial [16]. Magnetic resonance imaging-proton density fat fraction (MRI-PDFF) is a validated technique recommended for the early phase clinical trials that can assess hepatic steatosis in each segment of the liver and is more sensitive than liver biopsy at detecting subtle changes in hepatic steatosis [17]. Notably, there is a paucity of data, demonstrating that the severity of hepatic steatosis predicts outcomes, although recent data suggest elevated hepatic steatosis determined by MRI-PDFF is associated with a higher likelihood of fibrosis progression in patients with NAFLD [18]. Nonetheless, the relevance of a relative reduction in steatosis is unknown. Furthermore, steatosis may diminish and disappear with the onset of cirrhosis. Nevertheless, complete resolution (as opposed to a relative reduction) of hepatic steatosis in the non-cirrhotic patient is likely to be accompanied by improvement in NASH and better outcomes [19].

Elastography

Liver stiffness determined by elastography-based methods including vibration-controlled transient elastography (VCTE), magnetic resonance elastography (MRE), and shear-wave elastography (SWE) provides an accurate prediction of liver fibrosis and has potential as a surrogate end-point. Currently, however, VCTE has not been demonstrated to be dynamic to changes in liver fibrosis in NAFLD and up to one-third of NAFLD patients with a high VCTE reading will have normal results on repeated examination, limiting its utility as a clinical trial end-point [20]. MRE has greater accuracy across different fibrosis stages when compared with VCTE [21]. The accuracy of MRE for predicting fibrosis change over a 6 month period in a phase II trial examining selonsertib was also greater than VCTE but remained modest (AUC 0.62) [22]. MRE had 67% sensitivity, 64% specificity, 48% positive predictive value, and 79% negative predictive value for fibrosis improvement on biopsy. Inclusion of baseline MRE value increased the AUC to 0.79, suggesting that it may be a useful surrogate end-point, however, requiring further validation.

Surrogate drug treatment end-points: biochemical

Given the difficulty in conducting trials using paired liver biopsies, the use of validated surrogate biomarkers as end-points is critically needed. A number of serum-based biomarkers of fibrosis including the NAFLD fibrosis score (NFS), the FIB-4 index, Fibrometer^{V2G} and Hepascore, as well as transient elastography have been demonstrated to be prognostic of liver-related mortality in NAFLD [23]. Overall, these non-invasive fibrosis tests are useful at predicting the absence of fibrosis; however, they have modest ability to predict fibrosis, thereby limiting their usefulness as surrogate end-points. There is also limited information on the dynamic change of these measures in response to changes in fibrosis over time. Studies examining the change in the NFS, FIB-4, and AST to platelet ratio index (APRI) in response to lifestyle change or obeticholic acid therapy suggest that the accuracy of these models to predict fibrosis response is only modest [24, 25]. A model based on change in platelet count, HbA1c, and normalization of ALT developed in the same population was highly accurate at predicting fibrosis improvement or worsening (AUC 0.96); however, it requires external validation particularly in response to pharmacotherapy [24].

Mac-2-binding protein (Mac-2bp) and Wisteria floribunda agglutinin (WFA)-positive Mac-2bp (WFA$^+$-M2BP) have been identified as accurate serum markers of NASH and fibrosis in Asian and Caucasian populations [26, 27]. However, the accuracy of WFA$^+$-M2BP to predict change in liver inflammation, ballooning, and fibrosis over 48 weeks is low (AUC < 0.60) [28]. Confirmation of the accuracy of Mac-2bp to predict change in liver histology over time is needed before use as a clinical trial end-point.

Additional potential surrogate serum biomarkers of NASH include cytokeratin-18 (CK-18) fragments, serum ALT, adipocytokines, inflammatory mediators (e.g., c-reactive protein, TNF, IL-6, and CXCL10) and lipid products. Unfortunately, these markers have generally been disappointing as predictors of NASH or its improvement or have had limited validation. The limited accuracy of these biomarkers may reflect the generally low level of inflammation and liver injury in NASH as well as the heterogeneity of pathways of liver injury in this condition. Nevertheless, EASL treatment recommendations suggest serum ALT as a monitoring end-point for lack of efficacy, recommending that drug treatment be ceased if there is no reduction in serum ALT within 6 months of therapy among patients with initially elevated ALT levels [29]. Improvement in serum ALT tends to reflect alterations in liver steatosis and inflammation but not necessarily with fibrosis. Over the longer term, serum ALT levels tend to fall spontaneously as steatosis and inflammation reduces, but as fibrosis progresses. Thus, serum transaminases may be falsely reassuring and are not a suitable long-term end-point for treatment of NAFLD.

Conclusions

Efficacious drug therapy for patients with fibrotic NASH is urgently required to prevent the increasing burden related to this disease. Current end-points assessing drug treatment efficacy being utilized in phase 3 trials focus on improvement in the histological features of fibrosis and NASH. Simultaneously, long-term follow-up is required to assess whether these surrogate histological end-points parallel changes in hard end-points such as liver decompensation and liver-related death. In addition to effective drug treatment, there is an urgent need to develop validated and accurate non-invasive measures of drug efficacy in NASH.

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Compliance with ethical standards

Conflict of interest I declare that I have no conflict of interest.

Ethical approval This article is a review of the literature and does not contain any studies with human participants or animals performed by any of the authors.

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